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6. AUTHOR(S)

Robert Blake II

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Meharry Medical College
1005 D.B. Todd Blvd.
Nashville, TN 372088. PERFORMING ORGANIZATION
REPORT NUMBER

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13. ABSTRACT (Maximum 200 words)

The aims of this research are to study each of the various molecular mechanisms whereby toxic metal cations and oxyanions are chemically transformed by bacteria that live in the soil. The research effort for the current year has focused on the Xanthomonas-dependent transformations of selenium and lead. Conversion of selenite and ionic lead into insoluble biocolloids was found to occur widely in the genus Xanthomonas. The resulting biocolloids were shown to bear negative charges derived from biological polymers associated with the metals in the colloid. The formation of a lead biocolloid was accomplished from a lead-citrate complex where citrate was the sole carbon source for the bacterium. These observations could prove useful for the eventual exploitation of Xanthomonas and related genera for the removal of toxic wastes from selected, heavily polluted sites.

14. SUBJECT TERMS

Bioremediation, selenium, lead, colloids

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A. RESEARCH OBJECTIVES

The aims of this project are to study each of the various molecular mechanisms whereby toxic metal cations and oxyanions are chemically transformed by bacteria that live in the soil. The specific aims for the current grant period are as follows:

(1) To perform detailed kinetic studies on selected transformations using suspensions of Xanthomonas maltophilia strain OR-02;

(2) To identify, separate, purify, and reconstitute the minimum cellular components necessary for individual metal transformations by strain OR-02;

(3) To investigate the range of insoluble metal oxides that will serve as terminal electron acceptors in the anaerobic metabolism of Bacillus polymyxa strain D1; and

(4) To identify, separate, purify, and reconstitute the minimum cellular components necessary for metal oxide reductions by strain D1.

B. STATUS OF THE RESEARCH EFFORT

1. Specific Aims 1 and 2

Bacterial-dependent transformations of soluble metals are widespread in the genus Xanthomonas.

It is now recognized that bacterial strains formerly designated as Pseudomonas maltophilia (such as strain OR-02) are now more appropriately classified as Xanthomonas maltophilia. Having encountered scattered reports of metal-transformation activities in the genus Xanthomonas, we became suspicious that such activities were actually more widespread in this genus and species than previously realized. To test this notion, we acquired 15 strains of X. maltophilia from the ATCC and various colleagues both in this country and Canada. The strains were chosen to provide a cross section of the species; care was taken to select members that were initially isolated from a variety of diverse habitats. We were surprised to discover that each strain was capable of electrochemically reducing selenite to elemental selenium and of transforming and immobilizing soluble lead as a brown-black precipitate. Four of the strains electrochemically reduced mercuric ions to elemental mercury. Three of the strains (not the same ones that reduced Hg^{2+}) electrochemically reduced chromate to Cr(III).

The availability of a stable of organisms that share a fundamentally common pathway of iron respiration may provide opportunities to study a particular portion of the pathway in some of the members that proves to be intractable in others. For example, a key protein that is extremely difficult to isolate from one organism may prove to be easily obtained in good yield from another. On the other hand, detailed comparisons among different

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organisms that expressed different mechanisms for metal transformation may provide the opportunity to deduce the advantages and disadvantages of each. Although strain OR-02 is the most extensively characterized member of this group of bacteria, that does not mean that it will prove to be the predominant or most useful organism in any or all of the eventual bioremedial processes that may be developed. The need for basic information regarding the energy metabolism of all of these organisms is evident.

The biocolloids formed with selenium and lead were investigated by particle-analysis techniques.

The fine particulate matter formed when strain OR-02 is exposed to selenite or ionic lead was investigated with a DELSA 440 electrokinetic analyzer. This instrument quantifies colloid migration in an electronic field established by the application of a DC voltage. Electrophoretic motion is detected by the Doppler shift in frequency of scattered coherent light using a heterodyne method. An investigation of the red colloidal elemental selenium produced when strain OR-02 is grown in the presence of selenite revealed that a colloid of discrete size (200 to 400 nanometers) and charge (a net negative charge corresponding to around 3 to 4 mV of zeta potential) was produced. The colloid was stable over a pH range of 4 to 9, not disrupted by sonic oscillation, and persisted in culture fluid for months with no apparent change in morphology. The negative charge must arise from biological materials tightly associated with the red elemental selenium. Since the gray amorphous form of selenium is thermodynamically more stable than the red amorphous form, it follows that the biological materials associated with the selenium biocolloid contributes to its stability.

The formation of lead biocolloids by bacterial action produced a colloid smaller in size than the selenium biocolloid (only 50 to 150 nanometers in diameter) but one that displayed considerably more negative charge on its surface (corresponding to around 30 mV of zeta potential). Further electron microscopic studies revealed that the lead biocolloid included an extracellular matrix and that the lead appeared to be encapsulated in biological material. The presence of biological material at the surface of the biocolloid could account for the electrokinetic properties of the colloid and its similarities to those of the intact bacterial cells (which are also negatively charged).

A clue as to the nature of the extracellular matrix around these biocolloids was provided by the observation that growth of strain OR-02 in the presence of 10 mM 2-deoxyglucose (which inhibits the biosynthesis of complex polysaccharides) completely inhibited lead biocolloid formation. Further characterization of these biocolloids and efforts to identify the biological portions of the particles are in progress. One promising observation is that toxic cations at concentrations of 0.1 mM or less have little or no impact on the overall charge of the biocolloid. This is important to possible bioremediation strategies because biocolloid formation and subsequent separation activities may occur in environments where mixed toxic wastes occur.

Solubilization of toxic metals with citrate may provide a means to bioremediate selected solid wastes.

We have demonstrated that strain OR-02 produces a lead biocolloid when grown on a lead-citrate complex as its sole carbon source. The biological degradation of a metal-citrate complex is a promising observation because it may provide a means to solubilize and recover metals from complex solid wastes. Citric acid forms different types of complexes with the transition metals, lanthanides, and actinides. Such complexes can involve bidentate, tridentate, binuclear, or polynuclear complex species. Citrate could be exploited to extract metals such as Cd, Cr, Zr, Ni, and radionuclides such as Sr, Th, and U from solid wastes and liquid concentrates by forming water soluble metal-citrate complexes. Subsequent biodegradation of the metal complexes might permit recovery of the metal in a concentrated form within the bacterial biomass. Efforts to demonstrate the biodegradation of other metal-citrate complexes (especially those of uranium(VI)) are in progress.

Specific Aims 3 and 4

Preliminary experiments indicated that direct contact between *Bacillus polymyxa* strain D1 and insoluble manganese dioxide was necessary for detectable metal oxide reduction and manganese(II) solubilization to occur. We thus restricted our search for biological manganese dioxide reducing agents to macromolecules on the cell surface of the bacterium. The membrane fraction of strain D1 disrupted by various means contained conspicuous quantities of c-type cytochromes. Our working hypothesis is that one or more of these membrane-associated c-type cytochromes faces toward the outside of the cell and affects the facile reduction of insoluble manganese dioxide by direct contact. Efforts to achieve the reduction of manganese dioxide using a partially purified, detergent-solubilized membrane preparation have been hampered by the facile auto-oxidation of the cytochromes. We are currently assembling an anaerobic environment sufficient to create, stabilize, and manipulate electrochemically reduced cytochrome to test its efficacy in manganese dioxide solubilization studies.

C. PUBLICATIONS

\ (i) Published - three

"Chemical transformation of toxic metals by a *Pseudomonas* strain from a toxic waste site"; R.C. Blake, D.M. Choate, S.H. Bardhan, N.H. Revis, L.L. Barton, and T.G. Zocco; Environmental Toxicology and Chemistry, in press

"Application of biotechnology in management of industrial wastes containing toxic metals"; L.L. Barton, F.A. Fekete, L.O. Sillerud, C.J. Pigg, and R.C. Blake; Radioactive Waste Management and the Nuclear Fuel Cycle Journal, in press

"Biocolloid formation: an approach to bioremediation of toxic metal wastes"; L.L. Barton, H.E. Nuttall, W.C. Lindemann, and R.C. Blake; in Handbook of Bioremediation (D.L. Wise and D.J. Trantolo, Eds.) Marcel Dekker, New York, in press

(ii) Submitted - one

"On the microbial-dependent transformation of toxic metals: mechanism of selenite reduction by Pseudomonas maltophilia"; R.C. Blake, D.M. Choate, S.H. Bardhan, N.H. Revis, and J.H. Jackson; submitted to the Journal of Biological Chemistry

(iii) In preparation - none

D. PROFESSIONAL PERSONNEL

(i) Postdoctoral Associate - Dr. Gary Howard, employed for 9 months

(ii) Research Assistant - Elizabeth Shute, employed for the last 12 months

(iii) Graduate Students - Ken Williams (the recipient of an AASERT fellowship), William Baker, and Clarence Lewis

E. COUPLING ACTIVITIES

(i) Meeting presentations - none

(ii) Consultations - two invited seminars at the following institutions: Case Western Reserve University, Cleveland, OH; and University of Alabama, Tuscaloosa, AL

F. NEW DISCOVERIES

That the ability to chemically transform and immobilize certain toxic metals is widespread in the species Xanthomonas maltophilia.

That the colloidal bodies generated in the presence of selenite or lead have associated biological materials that impart a negative charge on the surface of the colloid. This property may be exploited to collect and separate the bioprecipitated metals.